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ORIGINAL ARTICLE

Effects of single and combined application of anti-cancer drugs on cervical adenocarcinoma

I. Antitumor activity *in vitro*

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Background. Establishment of effective combination chemotherapy regimen for patients with an adenocarcinoma of the uterine cervix has been long-awaited because there has been no documentation concerning chemosensitivity of this tumor against conventional antitumor agents.
Methods. To search for an effective combination regimen, 15 conventional antitumor drugs were tested for growth inhibitory effects on five different cervical adenocarcinoma cell lines.
Results. Etoposide, mitomycin C, adriamycin, epirubicin, and vinblastine, were singularly effective. Effects of combination chemotherapy were also tested using the above five antitumor agents plus interferon-γ. Etoposide, mitomycin C, and interferon-γ were the most effective when given in combination.
Conclusions. Combined treatment with the above three drugs seems worthy of consideration for clinical application.

Key words: adenocarcinoma; cervical carcinoma; combination chemotherapy; interferon-γ; *in vitro*

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Synopsis

VP-16 and MMC were found to be the most effective of 15 conventional antitumor agents for combination treatment of cervical adenocarcinoma, determined using cell lines.

Introduction

Adenocarcinoma, including adenosquamous carcinoma, represents only about 5–10% of all primary carcinoma of the cervix (1, 2), but this percentage is gradually increasing (3, 4, 5, 6). The incidence of squamous cell carcinoma is decreasing because most cervical carcinomas of this histology have a definite premalignant state and can be readily diagnosed and treated. On the other hand, the incidence of adenocarcinoma remains unchanged because it is difficult to recognize the premalignant state (7, 8, 9). It has been suggested that adenocarcinoma of the uterine cervix tends to metastasize earlier to lymphnodes and is less sensitive to radiation therapy and to chemotherapy than is squamous cell carcinoma (5, 10, 11, 12, 13, 14, 15). Radical surgery seems to be the treatment of choice for this tumor (10, 11, 12), however, treatment for advanced or recurrent cases has been most often unsuccessful. Therefore, we still have to rely upon the irradiation for the treatment of these cases. We found no documentation concerning chemosensitivity of adenocarcinoma of the uterine cervix against conventional antitumor agents.

In our series of *in vitro* and *in vivo* experiments using various gynecologic tumor cell lines, we observed that lines derived from cervical carcinoma were the most sensitive to IFN-γ and this agent was effective against both squamous cell carcinoma and adenocarcinoma (16, 17).

The present study was initiated to search for ef-

fective, conventional antitumor agents and to examine the effects of combinations of these agents, including IFN-γ, on five cervical adenocarcinoma cell lines.

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Cells. We used five human cervical adenocarcinoma cell lines (HeLa, OMC-4, CAC-1, TMCC-1, and JSK/CA-1) in this study. Cell lines OMC-4, CAC-1 and TMCC-1 were established by Dr. T. Yamada (Osaka Medical College, Osaka, Japan), Dr. O. Hayakawa (Sapporo Medical College, Sapporo, Japan) and Dr. M. Sakamoto (Tokyo Medical College, Tokyo, Japan), respectively. These cell lines were derived from well, moderate, and poorly differentiated adenocarcinoma of the endocervical type, respectively. Cell line JSK/CA-1 was established by Dr. H. Sasaki (Jikei University School of Medicine, Tokyo, Japan) and was derived from a well differentiated adenocarcinoma of the endometrioid type.

Cell cultures. All cell lines were passaged at intervals of 7–14 days and were cultured in Eagle's minimal essential medium (MEM) supplemented with 20% fetal calf serum (FCS), 100 U/ml of penicillin, 100mg/ml of kanamycin and 100 mg/ml amphotelicin B.

Interferon and Chemicals. Human recombinant Interferon-γ (IFN-γ) was provided by Toray Industries (Tokyo, Japan) and contained 1×10^6 IU/ml.

Fifteen conventional antitumor agents, etoposide, cisplatin, and carboplatin (VP-16, CDDP, and CBDCA, Bristol-Myers Squibb Co. Ltd, Tokyo, Japan), adriamycin, mitomycin-C, and 5-fluorouracil (ADM, MMC, and 5-FU, Kyowa Hakko Kogyo Co. Ltd, Tokyo, Japan), vinblastine and vincristine (VLB and VCR, Shionogi & Co. Ltd, Osaka, Japan), bleomycin and peplomycin (BLM and PEP, Nippon Kayaku Co. Ltd, Tokyo, Japan), epirubicin (EPIR, Farmitaria Carloerba Co. Ltd, Tokyo, Japan), aclarubicin (ACR, Yamanouchi Pharmaceutical Co. Ltd, Tokyo, Japan), actinomycin D (ACT-D, Banyu Pharmaceutical Co. Ltd, Tokyo, Japan), methotrexate (MTX, Ledele (JAPAN), Ltd. Tokyo, Japan), and cyclophosphamide (CPA, Shionogi & Co. Ltd, Osaka, Japan), were used for estimation of the growth-inhibitory activity of these cell lines. We used 40487S, an active metabolite of CPA, instead of CPA itself because CPA is not active without conversion in the liver. Four drug concentrations were used 0.01×, 0.1×, 1× and 10×Peak Plasma Concentration (PPC). Each PPC of 15 agents were based on information provided by the drug maker.

Assays. To examine the growth-inhibitory activity of a single agent, 2×10^4 cells of each cell line were seeded in separate plastic Petri dishes (35 mm in diameter; Falcon Plastics, Oxford, CA) and incubated at 37°C in a humidified atmosphere containing 5% CO_2 in air, followed by harvesting in 0.025% trypsin-EDTA on subsequent days. IFN-γ was added to the Petri dishes at the same time of cell seeding and the other drugs were added after four days of incubation. Cell numbers were counted two days after administration of the drugs. The number of cells was expressed as the mean, calculated for triplicate cultures at each concentration, and the growth inhibitory activity was expressed using the following formula.

$$\%\ \text{Growth Inhibition} = \frac{\text{mean cell number with drug}}{\text{mean cell number without drug}}$$

IC50 (Drug concentration required for 50% growth inhibition) was read from the growth inhibitory curve and was compared with PPC.

To examine the combination effect of the two drugs, the concentration of each drug used was 0.1×PPC. In case of IFN-γ, the concentration of 10 IU/ml was adopted for this study because clinically this concentration can be maintained with a daily intramuscular administration of the usual dosage.

Results

Anticellular activities of 15-drugs. Fig. 1 shows the anticellular effects of fifteen drugs on the growth of five cervical adenocarcinoma cell lines, respectively. To generalize the effects of different antitumor agents, the drug concentration was expressed as a relative value to PPC. IC50 of each drug for each cell line is obtained from each curve. In Fig. 1-1, IC50s of VP-16 for HeLa, CAC-1, JSK/CA-1 and TMCC-1 were all less than 0.1×PPC, but that for OMC-4 was higher than 0.1×PPC. IC50s of the other drugs were also obtained, in the same manner (Figs. 1-2 ~ Fig. 1-15, Table 1). Five antitumor agents, VP-16, ADM, EPIR, MMC, and VLB, which showed higher antitumor effects on many of these five cell lines, were selected for further investigation (Table 1).

Antitumor effects of these five drugs were tested in combined use. There were 10 combinations when two drugs were selected and all these combination treatments were used for the five cell lines. Percent growth inhibition by a single agent and by

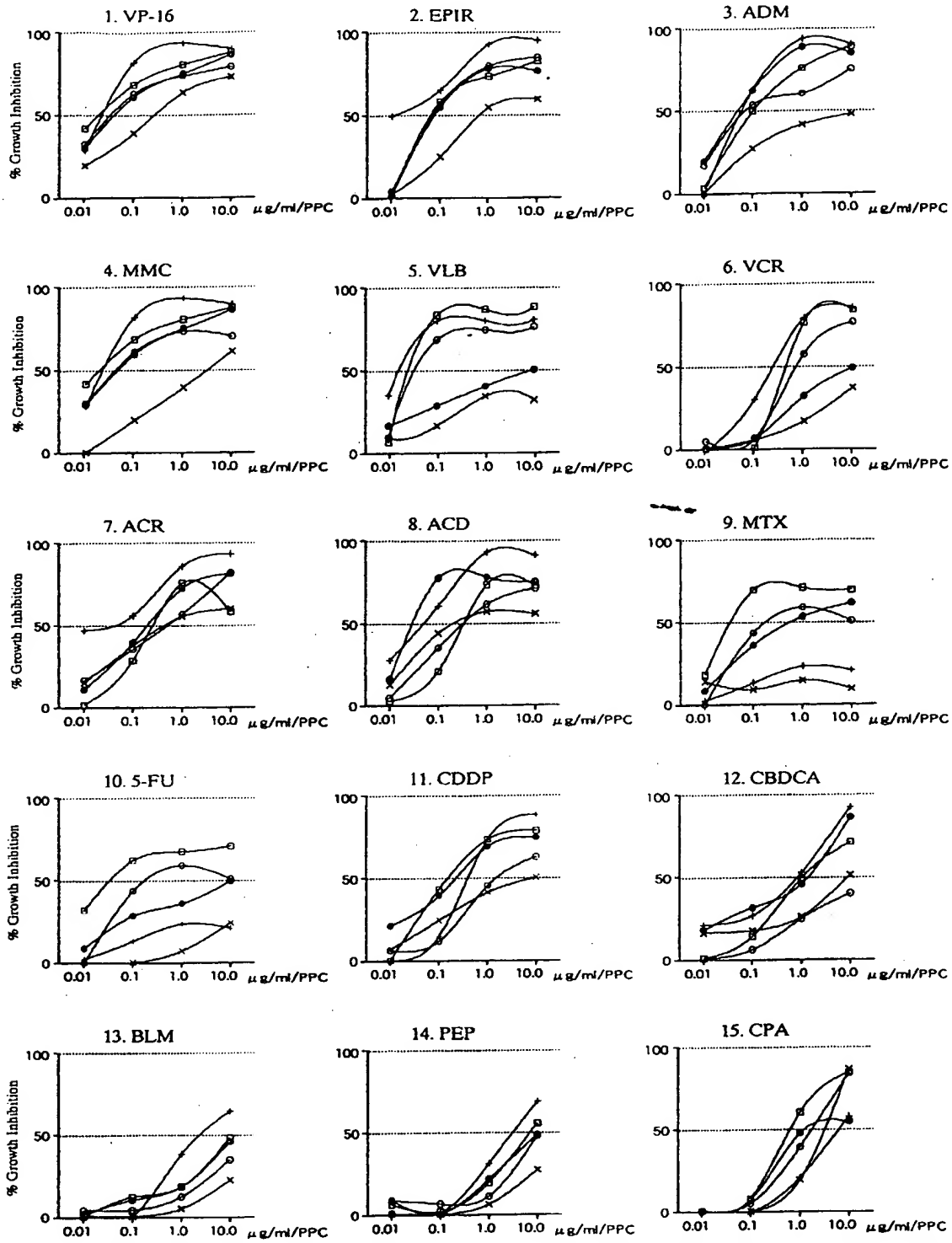

Fig. 1. Growth inhibitory activity of 15 antitumor agents against five cervical adenocarcinoma cell lines. Antitumor effects were expressed as % growth inhibition and drug concentration was expressed as relative value to PPC (Peak Plasma Concentration). IC50 (Drug concentration required for 50% growth inhibition) of each antitumor agent against each cell line was obtained from these growth inhibitory curves. (o: TMCC-1, □: CAC-1, ●: JSK/CA-1, x: OMC-4, +: HeLa). (*See Method for details*).

Table I. Effects of antitumor drugs on cervical adenocarcinoma cell lines

Drug	PPC	HeLa	TMCC-1	CAC-1	JSK/CA/1	OMC-4
VP-16	(20.0)	0.024	0.034	0.018	0.038	0.27
EPIR	(0.5)	0.0135	0.08	0.068	0.076	0.66
ADM	(0.4)	0.084	0.074	0.105	0.05	(−)
MMC	(1.0)	0.066	0.048	0.02	0.8	3.2
VLB	(0.1)	0.021	0.058	0.033	9.0	(−)
VCR	(0.01)	0.35	0.68	0.45	(−)	(−)
ACR	(0.6)	0.0135	0.4	0.22	0.185	0.41
ACD	(0.1)	0.255	0.36	0.21	0.032	0.21
MTX	(1.0)	(−)	0.2	0.042	0.6	(−)
5-FU	(10.0)	4.8	(−)	0.03	(−)	(−)
CDDP	(2.0)	0.37	3.0	0.16	0.22	7.6
CBDCA	(10.0)	0.85	(−)	1.1	1.12	9.8
BLM	(1.0)	2.7	(−)	(−)	(−)	(−)
PEP	(1.0)	3.4	(−)	7.2	(−)	(−)
CPA	(3.0)	0.4	1.4	0.56	1.7	3.4

Note: Numbers within parenthesis indicated Peak Plasma Concentration (μg/ml) (PPC) and antitumor effects were expressed as IC50 relative to PPC. Less than 50% of growth inhibition was expressed as (−). Calculated relative values less than 0.1 were underlined.

Table II. Growth inhibitory effects of combination treatment against TMCC-1

Drug A	Drug B	%Growth inhibition by Drug A alone	% Growth inhibition by Drug B alone	% Growth inhibition by combination of A and B
VP-16	MMC	71.85	68.50	77.85
MMC	VLB	68.50	64.02	73.59
VP-16	EPIR	71.85	65.99	72.48
VP-16	ADM	71.85	62.07	71.78
EPIR	MMC	65.99	68.50	71.69
ADM	MMC	62.07	68.50	71.68
VP-16	VLB	71.85	64.02	71.02
ADM	EPIR	62.07	65.99	65.02
ADM	VLB	62.07	64.02	62.26
EPIR	VLB	65.99	64.02	54.88

a combination of the two against TMCC-1 cell line is listed in Table II, in the order of the better combination. Combination of VP-16 and MMC was the most effective and combinations of MMC+VLB and VP-16+EPIR were also more effective than the other combinations. Similarly, the best three combinations were determined against all the cell lines (Table III). Cumulative numbers of appearance of each drug are shown in the lower column of Table III, which suggests that the best two drugs are VP-16 and MMC.

On the other hand, IFN-γ was examined for its antitumor effect when combined with five respective antitumor agents. As shown in Table IV, the most suitable effect was obtained when cells were treated in combination with VP-16.

Discussion

Establishment of effective treatments for patients with an adenocarcinoma of the uterine cervix has been long-awaited because radiation therapy and chemotherapy are not so effective for this histology type. Previous studies showed that different tumors with the same histology type respond differently to the same chemotherapy. Therefore, ideally, the most effective antitumor agents should be selected following a chemosensitivity test for the treatment of an individual tumor (18). However, it is not practical for routine clinical use because it is not time and cost effective and the success rate is low (usually less than 50%).

Chemotherapy regimen for the treatment of cervical adenocarcinoma has been basically decided according to the combination chemotherapy used for other adenocarcinomas, especially for ovarian carcinomas. Therefore, the standard chemotherapy regimen is composed mainly of cisplatin together

Table III. The best three combinations for the treatment of each cell line

	TMCC-1	OMC-4	JSK/CA-1	CAC-1	HeLa
1	VP16+MMC	MMC+VLB	VP16+VLB	ADM+EPIR	VP16+EPIR
2	MMC+VLB	VP16+MMC	VP16+ADM	EPIR+MMC	VP16+ADM
3	VP16+EPIR	ADM+VLB	VP16+EPIR	ADM+MMC	EPIR+MMC

Note: Cumulative numbers of appearance of each drug are as follows. VP-16: 8, MMC: 7, EPIR: 6, ADM: 5, VLB: 4.

Table IV. The effective combinations with IFN-γ

	TMCC-1	OMC-4	JSK/CA-1	CAC-1	HeLa
1	IFN+VP16	IFN+VP16	IFN+VP16	IFN+VLB	IFN+EPIR
2	IFN+MMC	IFN+MMC	IFN+EPIR	IFN+VP16	IFN+VLB

Note: Cumulative numbers of appearance of each drugs are as follows. VP-16: 4, MMC: 2, EPIR: 2, VLB: 2.

with anthracycline and cyclophosphamide. However, we find no documentation of the successes of these regimens. We reported that IFN-γ has a higher antitumor activity against cervical carcinoma cell lines, derived from both squamous cell carcinoma and adenocarcinoma (16, 17), and *in vivo* experiments suggested the possibility of clinical use (17, 19, 20). Our present study revealed that VP-16 and MMC were the most effective antitumor agents among fifteen tested conventional drugs, in both singular and combined use and that IFN-γ also showed the highest combination effect with these two drugs. IFN-γ affects the cytoplasmic membrane and decreases membrane fluidity (21, 22, 23, 24, 25). This membrane modification might contribute to the increased antitumor effect, in cases of a combination with other antitumor agents.

In vivo experiments using transplanted tumors in nude mice revealed that combination chemotherapy with IFN-γ, VP-16, and MMC was the best combination for the treatment of cervical adenocarcinoma (manuscript in preparation). On the basis of the results obtained from the above *in vitro* and *in vivo* experiments, our chemotherapy regimen warrants consideration for possible clinical use.

Only the OMC-4 cell line showed resistence to chemotherapy. This line is derived from well differentiated adenocarcinoma of the endocervical type and has the longest doubling time among five cell lines (26). Furthermore, no HPV DNA was detected and a point mutation of p53 gene was demonstrated in this cell line, while HPV 16 or 18 DNA was detected and no p53 gene abnormality was observed in the other lines (27). The above observations suggest the possibility that the process of carcinogenesis in OMC-4 differs. Similar tumors may play in part for the morbid prognosis of cervical adenocarcinoma. To further improve the present rate of survival of subjects with cervical adenocarcinoma, new, effective treatment should be considered for this refractory tumor group.

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